Paragraphs 1 and 3 on page 73

(g) Quantitative Taqman RT-PCR

The same total RNA samples used for the GeneChip experiments can be analyzed using a *Taqman*® EZ RT-PCR kit. (PE Applied Biosystems) to confirm gene expression changes. Total RNA samples can be diluted to a concentration of 50ng/ul and a total of 50 ng can be used for each reaction. Primers and florescence probes for PSA and ID-1 and ID-3 can be designed using the Primer Express software and were chosen based upon the manufacturer's recommendations for primer selection. The primers used can be of 100uM concentration and for PSA can be PSA-F (forward primer) CGTGGCCAACCCCTGA (SEQ ID NO: 1), PSA-R (reverse primer) CTTGGCCTGGTCATTTCCAA (SEQ ID NO: 2), and PSA-P (probe) CACCCCTATCAACCCCCTATTGTAGTAAACTTGGA (SEQ ID NO: 3). For ~~SMARC1~~ SMARCD1 and ~~SMARC3~~ SMARCD3, primers can be designed based on the sequences of ~~SMARC1~~ SMARCD1 and ~~SMARC3~~ SMARCD3 available from Genbank Accession numbers U66617 and U66619, respectively.

A1

Samples can be prepared using a reagent mix of manufacturer supplied RT-PCR components [(5X TaqMan EZ Buffer, manganese acetate (25 mM), dATP (10mM), dCTP (10mM), dGTP (10mM) and dUTP (20mM), rT*th* DNA polymerase (2.5U/µl), AmpErase UNG (1 U/µl), primers (final concentration 1µM) and RNA (50ng)], following manufacturer's recommendations. In addition, GAPDH control samples for standard curve generation and subsequent quantitation of sample RNA can be prepared. Primers and probe for GAPDH were included in the kit (GAPDH forward and reverse primers 10µM, GAPDH probe 5 µM). β-actin can also be used for standard curve generation, and dilutions can be made for both genes that ranged from 5 X 10^6 copies to 5 X 10^1 copies. The assay can be performed on a Perkin-Elmer/Applied Biosystems 7700 Prism, and the PCR cycling parameters can be chosen based on the manufacturer's recommendations. RNA of samples can be normalized to GAPDH and β-actin and quantified.

(h) Western Blot Analysis

A2

To demonstrate that the protein production of SMARC (e.g., ~~SMARC1~~ SMARCD1 and/or ~~SMARC3~~ SMARCD3) can be regulated by androgen, Western blot analysis can be performed. For Western blot analysis, LNCaP cells can be plated in 6-well plate at 1 x 10^6 cells/well in

Paragraphs 1 and 2 on page 74

charcoal stripped serum containing medium. Cells can be treated with a suitable amount of androgen, e.g., 10 nM DHT and harvested at designated time. Cells can be harvested in MPER reagent (Pierce, Rockford, IL) containing 400 mM NaCl. Protein can be quantified by Bradford method (Bradford (1976) *Anal. Bioch.* 72: 248-254). A suitable amount of protein, e.g., 30 μg of protein can be electrophoresed on a 12% SDS-PAGE gel and transferred to a PVDF membrane using a Bio Rad liquid transfer apparatus. The PVDF membrane can be incubated in TBST (TBS with 0.1%Tween-20) with 3% milk for 15 minutes before the addition of the first antibody, e.g., rabbit anti-SMARC antibody (anti-~~SMARC1~~ SMARCD1, or anti- ~~SMARC3~~ SMARCD3 antibody). After overnight incubation, the PVDF membrane can be washed 3 times with TBST and incubated with a second antibody, anti-rabbit-IgG coupled with horseradish peroxidase (Transduction Labs) for one hour. The PVDF membrane can then be washed 3 times with TBST and protein can be detected by using an enhanced chemiluminescence detection system (Pierce).

A2

(i) Tissue Microarray Construction and Analysis

To investigate the role of SMARC, e.g., ~~SMARC1~~ SMARCD1 or ~~SMARC3~~ SMARCD3 in solid tumors, tissue microarray analysis can be performed on multiple human normal (*i.e.*, control samples) and prostate diseased specimens (Clinomics, Inc.). Following fixation in 10% neutral buffered formalin, tissues can be selected, trimmed, and placed in a processing cassette. The cassette can then placed in a processing basket on a Shandon Hypercenter™ tissue processor in which the tissues can be exposed to a series of buffers over a 16 hour processing cycle (10% Neutral Buffered formalin, 70%, 95%, 100% ethanol, xylene, and melted paraffin embedding media). All steps should be carried out under vacuum at 40°C except for the paraffin steps which should be at 58°C. Following processing, the tissues can be removed from the cassettes and embedded in paraffin blocks. The resulting blocks can be sectioned at 5 μm and mounted on glass slides. The slides can be heated at 58°C for 30 minutes prior to staining. Antibody α- SMARC (e.g., anti-~~SMARC1~~ SMARCD1 or anti-~~SMARC3~~ SMARCD3) can be titred to a suitable dilution, e.g., 1:150 dilution using DAKO® Antibody Diluent. Staining of test specimen can be performed employing HIER in pH 6.0 citrate buffer with no pretreatment. Tissues can then be stained using the Ventana ES® Automated Immunohistochemistry Stainer, involving the use of a standard indirect

immunoperoxidase protocol with 3,3'-diaminobenzidine as a chromagen. Grading of the A2 immunohistochemical staining is based on the intensity of the cytoplasmic staining of the

First full Paragraph on page 75

(j) Transient Transfection of COS cells

To determine the effect of SMARC, (e.g., ~~SMARC1~~ SMARCD1 and/or ~~SMARC3~~ SMARCD3) on the transcriptional activity of androgen receptor (AR), COS-1 cells can be transiently transfected with a reporter construct containing androgen receptor response element along with an expression vector encoding ~~SMARC1~~ SMARCD1 or ~~SMARC3~~ SMARCD3. COS-1 cells can be plated in 6-well plates at a density of 2 $x10^5$ cells per well in 2-ml pheno red-free DMEM containing 10% charcoal-stripped fetal bovine serum. The next morning, medium can be replaced with 2-ml DMEM. Indicated amount of DNA in 100 µl of DMEM can be mixed with 6 µl of PLUS reagent (Gibco) and incubated at room temperature while 4 µl of lipofectamine can be mixed with 100 µl of DMEM. After 30 min of incubation, the two mixtures can be combined together and added dropwise to each well. After incubation with DNA for 4 hours, 2 ml of phenol red-free DMEM containing 10% charcoal-stripped fetal bovine serum can be added and cells treated with indicated chemicals for additional 24 hours before being harvested.

A3

Paragraph 1 on page 78

Example 2: Screening for Compounds Useful for the Treatment of Prostate Cancer

The cDNA and protein sequences ~~SMARC1~~ SMARCD1 or ~~SMARC3~~ SMARCD3 are available in the public database Genbank with accession numbers, U66617 and U66619, respectively. The publications and sequence databases provide those skilled in the art with the genes needed to prepare the transfected cell lines useful in for the following screening assays.

Test compounds potentially useful for the treatment of prostate cancer can be identified by expressing ~~SMARC1~~ SMARCD1 or ~~SMARC3~~ SMARCD3 in prostate cancer cells (*e.g.*, LNCaP cells) which are stably transfected with a vector capable of expressing ~~SMARC1~~ SMARCD1 or ~~SMARC3~~ SMARCD3 in the presence of tetraceycline (Tet-on system, available from Clontech). The transfected LNCaP cells can be cultured under suitable conditions (*e.g.,* in T175 culture flasks in RPMI-1640 medium supplemented with 10%; fetal calf serum (FCS), 3mM L-glutamine, 100 μg/ml streptomycin, and 100 units/ml penicillin. To examine the effects of steroids, cells can be cultured for 2 days in RPMI 1640 medium containing 5% FCS pretreated with dextrancoated charcoal (CT-FCS). The cells can be incubated in the presence of a test compound with or without Tetracycline and the growth rate of the cells can be measured. A compound that demonstrates differential inhibitory activity in cells treated with Tet versus those not treated with Tet is a potential therapeutic compound for the treatment of prostate cancer.